Date of Approval: March 27, 2012

# FREEDOM OF INFORMATION SUMMARY

## SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 111-636

LINCOMIX

Lincomycin hydrochloride
Water soluble powder
Honey bees

For the control of American foulbrood (*Paenibacillus larvae*) in honey bees.

Sponsored by:

Pharmacia and Upjohn Co., a Division of Pfizer, Inc.

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## I. GENERAL INFORMATION:

| | |
|---|---|
| **A. File Number:** | NADA 111-636 |
| **B. Sponsor:** | Pharmacia and Upjohn Co.,<br>a Division of Pfizer, Inc.<br>235 East 42d St.<br>New York, NY 10017<br><br>Drug Labeler Code: 000009 |
| **C. Proprietary Name(s):** | LINCOMIX |
| **D. Established Name(s):** | Lincomycin hydrochloride |
| **E. Pharmacological Category:** | Antimicrobial |
| **F. Dosage Form(s):** | Water soluble powder |
| **G. Amount of Active Ingredient(s):** | 192 grams/bottle |
| **H. How Supplied:** | 16.92 oz (480 gm) bottles |
| **I. How Dispensed:** | Over-the-counter (OTC) |
| **J. Dosage(s):** | 100 mg per hive weekly for 3 weeks |
| **K. Route(s) of Administration:** | Oral – mixed with 20 g confectioners'/powdered sugar and dusted over the top bars of the brood chamber. |
| **L. Species/Class(es):** | Honey bees |
| **M. Indication(s):** | For the control of American foulbrood (*Paenibacillus larvae*) in honey bees. |
| **N. Effect(s) of Supplement:** | This supplement provides for the addition of a new species to the label for the control of American foulbrood (*Paenibacillus larvae*) in honey bees. |

## II. EFFECTIVENESS:

The data are summarized in Public Master File (PMF) 005-988 and contained in the publicly disclosable investigational new animal drug (INAD) file 010766 sponsored by National Research Support Project #7 (NRSP-7), a national agricultural research program funded by USDA for obtaining data in support of FDA approval for use of new animal drugs in minor species and for minor uses in major species. Based on the results of the dose confirmation study, NRSP-7 chose 100 mg lincomycin hydrochloride per hive weekly for three weeks as the labeled dosage regimen.

### A. Dosage Characterization:

In the dose confirmation study, the 100 and 200 mg/lincomycin hydrochloride per hive doses and the treatment regimens were chosen for evaluation based upon the dose and treatment regimens used in the lincomycin hydrochloride target animal safety and human food safety (residue depletion) studies, which were based on comparable zones of inhibition to oxytetracycline (OTC) in the microbiological assay [see Kochansky et al., Screening alternative antibiotics against oxytetracycline-susceptible and -resistant *Paenibacillus larvae.* Apidologie 32 (2001) 215-222].

The doses tested are also supported by the work of Dr. Mark F. Feldlaufer [see Feldlaufer et al., Lincomycin hydrochloride for the control of American foulbrood disease of honey bees. Apidologie 32 (2001) 547-554].

### B. Substantial Evidence:

**Dose Confirmation Study**

1. Title: "Effectiveness of lincomycin hydrochloride in the control of American Foulbrood Disease in honey bees." May to August 2001.

2. Investigator and Study Sites:

   The study was conducted at two apiary sites near Beltsville, MD by personnel from the USDA ARS Bee Research Laboratory.

   Principal Investigator: Mark F. Feldlaufer, USDA Agricultural Research Service, Bee Research Laboratory, Beltsville, Maryland

3. Study Design:

   a. *Objective:* To determine the effectiveness of lincomycin hydrochloride in control of the causative agent of American foulbrood (AFB: *Paenibacillus larvae*) of immature honey bees when applied in a dust of confectioners' sugar to honey bee colonies.

   b. *Test Animals*: Thirty colonies (hives) of honey bees (*Apis mellifera*) were established in two isolated apiaries (15 colonies per apiary). Each colony had to meet the following criteria:

- A viable laying queen
- Contain approximately 40,000 or more adult worker bees
- Have uncapped brood
- Have no visible signs of American foulbrood disease
- Contain no surplus honey

To initiate disease, all colonies were inoculated twice with a suspension of *P. larvae* spores 35 days apart. Eighteen days after the second inoculation, the colonies were rated for presence and severity of AFB as described under "*Measurements and Observations*" below.

c. *Test Article Administration*: The 30 honey bee colonies enrolled in the study were randomly assigned to one of three treatment groups at the two apiary sites, and treatment was begun on Day 58 (6 days after the colonies were rated for presence and severity of AFB). Assigned treatments are described in Table II.1.

Table II.1. Summary of treatment groups.

| Group | Treatment Regimen |
|---|---|
| Control | Control (confectioners' sugar) 20 g applied as a dust inside the bee colony three times at weekly intervals |
| 100 mg | Lincomycin hydrochloride at a rate of 100 mg mixed with 20 g of confectioners' sugar and applied as a dust inside the bee colony three times at weekly intervals (total dose of 300 mg/hive). |
| 200 mg | Lincomycin hydrochloride at a rate of 200 mg mixed with 20 g of confectioners' sugar and applied as a dust inside the bee colony three times at weekly intervals (total dose of 600 mg/hive). |

The bees ingested the sugar mixture to clean the hive. The worker bees then fed the bee larvae, thus treating them.

d. *Measurements and Observations:* The post-treatment colony disease evaluator was masked to treatment. All colonies were disease rated 18 days after the second AFB inoculation but prior to treatment on a scale of 0 to 3 based on a modification of the method proposed by Hitchcock *et al.* (1970). All hive frames were examined, and each frame with brood was rated as follows: 0= no signs of disease; 1= <10 cells per frame affected; 2= 11-100 cells per frame affected; 3= > 100 cells per frame affected. The bee colonies were rated again for AFB on the last treatment day (Day 73 of the study) and 46 days later (Day 119 of the study).

4. Statistical Analysis: The experimental design was a completely randomized design structure with a three-way (2x3x3) treatment structure (apiary, dosage, and time of treatment). All colonies were ranked for AFB severity into groups of three and then assigned to treatment within group. The experimental unit was a colony of bees (n = 30 colonies) and the response variable for each experimental unit was constructed by scoring each brood frame as to the severity of the AFB infection (0, 1, 2, 3) and calculating the average score. The data from the three scoring events (before treatment, last treatment day, and 46 days after the last treatment) were analyzed separately using the nonparametric test, Wilcoxon Rank Sum.

5. Results: All bee colonies completed the study (10 colonies each for the 100 mg, 200 mg, and control treatments). The average single colony score was calculated by dividing the total frame scores of each colony by the number of frames in that colony. The average colony scores in each treatment group were added and divided by 10 (the number of colonies in each treatment group) to yield a mean treatment score for that group.

   All treatment groups had comparable mean AFB severity ratings pretreatment. By the end of the study, AFB in the untreated group had increased considerably and the colony scores in the untreated control colonies had risen to a mean treatment group score of 0.738. By comparison, no colonies in the either the 100 mg or the 200 mg lincomycin-treated groups had active disease. Average colony scores by treatment group are presented in Table II.2.

   Table II.2. Average colony scores of AFB infected honey bee colonies by treatment (10 colonies per treatment).

| Treatment | Pretreatment Score | Day 73 Score (last day of treatment) | Day 119 Score (46 days after last treatment) |
|---|---|---|---|
| Control | 0.425 | 0.187 | 0.738 |
| 100 mg | 0.389 | 0.052 | 0.000 |
| 200 mg | 0.521 | 0.090 | 0.000 |

6. Adverse Reactions: No adverse events were reported during the study.

7. Conclusion: Based on the results of this study, lincomycin hydrochloride is effective in controlling American foulbrood (*Paenibacillus larvae*) when applied to infected honey bee colonies as a dust in confectioners' sugar, three times, one week apart at a rate of 100 mg or 200 mg lincomycin hydrochloride per colony per treatment.

## III. TARGET ANIMAL SAFETY:

The data are summarized in PMF 005-988 and contained in the publicly disclosable INAD file 010766, sponsored by NRSP-7.

### A. Toxicity Study

1. Title: "Toxicity of lincomycin hydrochloride to immature and adult honey bees." July to September 2000.

2. Principal Investigator: Mark F. Feldlaufer, USDA Agricultural Research Service, Bee Research Laboratory, Beltsville, Maryland

3. Study Design:

   a. *Objective*: To demonstrate the safety of lincomycin hydrochloride treatments to honey bee colonies, including adults, larvae, and the queen when administered at 0, 200, 600, or 1000 mg lincomycin hydrochloride per hive once weekly for 3 weeks.

   b. *Test Animals*: Twenty colonies of honey bees (*Apis mellifera*). All colonies were examined prior to inclusion in the study, and only healthy hives were used. Each colony had to meet the following criteria:

      - Have a viable laying queen
      - Contain at least 40,000 adult worker bees
      - Have uncapped brood
      - Have no visible signs of disease
      - Contain no surplus honey

   c. *Test Article Administration*: Water soluble powder formulation of lincomycin hydrochloride. The test article was mixed in 20 grams of confectioners' sugar. The preparation was dusted across the tops of the frames in the hive. Twenty honey bee colonies were enrolled in the study and randomly assigned to one of five dose groups as described in Table III.1 (four colonies per group). All groups were administered control or test articles weekly for nine weeks (3X the proposed duration of 3 weeks).

Table III.1. Summary of treatment groups.

| Group | Treatment Regimen (Weekly for Nine Weeks) |
|---|---|
| Negative control | No confectioners' sugar or test article applied |
| Sugar control | 20 g confectioners' sugar only |
| 200 mg | 200 mg lincomycin hydrochloride mixed with 20 g confectioners' sugar |
| 600 mg | 600 mg lincomycin hydrochloride mixed with 20 g confectioners' sugar |
| 1000 mg | 1000 mg lincomycin hydrochloride mixed with 20 g confectioners' sugar |

The bees ingested the sugar mixture to clean the hive. The worker bees then fed the bee larvae, thus treating them.

d. *Measurements and Observations*: Adult bee mortality, the presence of sealed brood (healthy larvae), and queen health were observed during the study.

*Adult honey bees*: Because honey bees remove dead adult bees from the hive, plastic fabric was placed in front of each hive. The number of dead adult bees was recorded one, four, and seven days after each treatment for the duration of the study (nine weeks). After the counts were made, the dead bees were removed and the fabric was replaced for the next count.

*Larval honey bees*: Two areas (100 cells each; approximately 25 $cm^2$ each) on a frame containing larval bees were marked in every colony immediately prior to treatment. Sealed brood were counted in each marked area 7 days after the week 1, week 4, and week 7 treatment administrations. The percentage of emerging and emerged adults in each marked area was recorded 18 days after the week 1, week 4, and week 7 treatment administrations.

*Queen*: Queens were marked at the beginning of the study for quick identification. At the end of the study, the queen was visually found and observed (Day 64).

4. Statistical Analysis:

The experimental unit for the analysis was colony. All data were analyzed using repeated measures analysis of variance. The counts of dead adult bees (Y) from each colony were transformed using log (Y+1) prior to analysis. The model for comparing counts of dead adult bees included dose group, observation day (1, 4, and 7 days after each treatment), and week (1 through 9) as fixed effects. The proportions (P) of larvae sealed 7 days after the week 1, week 4, and week 7 treatment administrations were transformed using the

arcsine transformation, $\sin^{-1}$(square root of P), prior to analysis. The model for comparing proportions of sealed larvae included dose group and treatment week (weeks 1, 4 and 7) as fixed effects. The presence of emerging adults and the presence of a viable queen at the end of the study were not statistically analyzed.

5. Results:

*Adult honey bees*: The data in the following table (Table III.2) represent 27 days (1, 4, and 7 days after each treatment over 9 weekly treatments) of collected dead bee counts. The statistical analysis did not show any significant differences due to dose group or observation day.

Table III.2. Adult Bees: Total dead in each treatment group.

| Group | Total Dead |
|---|---|
| Negative control | 320 |
| Sugar control | 292 |
| 200 mg | 459 |
| 600 mg | 369 |
| 1000 mg | 541 |

*Larval honey bees*: The data in the following table (Table III.3) represent the average percent of sealed brood in each area for all hives in each treatment group. The statistical analysis did not show any significant differences due to dose group although there was a significant change over time, where the proportions of sealed brood decreased in all groups.

Table III.3. Average percent sealed brood in each area for all hives by treatment group and observation number.

| Group | Obs 1 | Obs 1 | Obs 2 | Obs 2 | Obs 3 | Obs 3 |
|---|---|---|---|---|---|---|
| | Area 1 | Area 2 | Area 1 | Area 2 | Area 1 | Area 2 |
| Negative control | 95.75 | 97.25 | 89.66 | 95 | 68.5 | 45 |
| Sugar control | 93.5 | 91 | 87.25 | 92 | 89.75 | 67.5 |
| 200 mg | 98.5 | 98.25 | 93 | 94 | 85.25 | 85.33 |
| 600 mg | 97.5 | 95.5 | 93.5 | 95.5 | 91.75 | 64 |
| 1000 mg | 75.25 | 92 | 95 | 95 | 67.5 | 67 |

*Emerging adults*: In some parts of the study, there was no sealed brood to mark because all adults were emerged, and therefore also there were no emerging adults to count. There was no sealed brood to mark in: 1) one

area of one hive and in both areas of another hive in the negative control group, 2) one area of one hive in the sugar control group, 3) one area of one hive in the 200 mg group, 4) one area of one hive in the 600 mg group, and 5) both areas of one hive in the 1000 mg group. Among the countable brood, Areas 1 and 2 for all treatment groups had 100% emergence of adult bees with the exception of Area 1 after the first observation in the 1000 mg group which had 75% emergence of adult bees.

*Queens*: No queens were replaced during the study. On Day 64, all queens were accounted for.

6. Conclusion: The data demonstrated that lincomycin hydrochloride is safe when administered to honey bee colonies at a level of 200 mg per hive once weekly for 3 weeks. During the study no adverse effects associated with the drug product were seen.

## IV. HUMAN FOOD SAFETY:

### A. Microbial Food Safety (Antimicrobial Resistance)

The Agency determined that there is low risk to human health from the use of lincomycin for the control of American foulbrood (*Paenibacillus larvae*) in honey bees when administered at 100 mg lincomycin/hive once weekly for 3 weeks. Humans are not commonly infected with bacteria of public health concern associated with bees or honey, and lincomycin-resistant bacteria from honey have not been known to pose a serious public health threat. An overall risk estimation of low allows over-the-counter marketing status and high extent of use. These risk management strategies are compatible with the approved conditions of use for lincomycin. Based upon information contained in PMF 005-988, the Agency has determined that the requirements for microbial food safety with respect to antimicrobial resistance have been satisfied.

### B. Impact of Residues on Human Intestinal Flora

The agency agrees with the Joint European Committee on Food Additives (JECFA) evaluation of the safety of lincomycin residues with respect to their effects on human intestinal flora[1]. The decision-tree approach followed in the JECFA evaluation is similar to, and incorporates the same concepts as the pathway approach proposed by the agency for assessing the safety of antimicrobial drug residues in food. Since the drug reaches the colon and remains microbiologically active following oral intake, the safety of residues on the intestinal flora needed to be demonstrated. The human food safety of lincomycin residues was assessed using a human study performed with clindamycin.

The agency carefully reviewed and agrees with the JECFA report and approach, and considers the human study to be appropriate for determining the microbiological ADI (mADI) for lincomycin residues, because it was performed in a closely related drug (clindamycin) and with a sufficient number of individuals

[1] WHO/JECFA monographs:
http://www.who.int/foodsafety/chem/jecfa/publications/monographs/en/index.html
http://www.inchem.org/documents/jecfa/jecmono/v45je02.htm

dosed for a prolonged period of time. The calculation of the mADI considered the NOEL obtained in the study, and a safety factor that accounted for inter-individual variation and for higher bioavailability of lincomycin in the colon compared to clindamycin.

The agency concludes that the established toxicological ADI of 25 µg/kg body weight/day protects against adverse effects on human intestinal flora. Consequently, the ADI for lincomycin residues remains as 25 µg/kg body weight/day.

## C. Toxicology

CVM did not require toxicology studies for this supplemental approval. The FOI Summary for the original approval of NADA 111-636, dated January 23, 1990, contains a summary of all toxicology studies. The FOI Summary was made available January 31, 1990 (55 FR 3208-3209).

## D. Assignment of the Final ADI:

No reassessment of the toxicological ADI was necessary. The agency concludes that the established toxicological ADI of 25 µg/kg body weight/day protects against adverse effects on human intestinal flora; therefore, the final ADI for lincomycin residues remains as 25 µg/kg body weight/day. The FOI Summary for the original approval of NADA 111-636, dated January 23, 1990, contains a summary of all toxicology studies. The FOI Summary was made available January 31, 1990 (55 FR 3208-3209).

## E. Safe Concentrations for Total Residues (edible tissues and injection sites, if applicable):

Not Applicable.

## F. Residue Chemistry:

### 1. Summary of Residue Chemistry Studies

The data are summarized in PMF 005-988 and contained in the publicly disclosable INAD file 010766, sponsored by NRSP-7.

a. Type of Study: Residue depletion study of lincomycin hydrochloride in honey

b. Investigator: Mark F. Feldlaufer, USDA Agricultural Research Service, Bee Research Laboratory, Beltsville, Maryland

c. Study Dates: February 23 to April 5, 2001

d. Test Animals: Honey bee, *Apis mellifera*

e. Number of Animals: 40,000 workers plus queens/colony; 12 colonies

f. Route of Administration: Dusting of hive with lincomycin-containing confectioners' sugar

g. Treatment Groups:

1) Untreated controls (4 colonies)
2) 200 mg lincomycin in 20 g confectioners' sugar (1X; 4 colonies)
3) 1000 mg lincomycin in 20 g confectioners' sugar (5X; 4 colonies)

h. Duration of Treatment: Once every seven days for a total of three treatments (21 days).

i. Sampling: Honey was sampled as indicated in Tables IV.1 and IV.2.

j. Results:

Table IV.1. Mean concentrations (in ppm) of lincomycin in brood chamber honey (lower, upper 95% confidence limits).

| Treatment | 7 days after final treatment | 14 days after final treatment | 20 days after final treatment |
|---|---|---|---|
| 200 mg (1x) | 1.22 (0.73, 2.12) | 0.65 (0.40, 1.09) | 0.41 (0.26,0.67) |
| 1000 mg (5x) | 13.94 (7.15, 29.80) | 2.41 (1.39, 4.31) | 1.57 (0.92, 2.73) |
| 0 mg (control) | 0.39 (0.25, 0.64) | 0.15 (0.10, 0.23) | 0.12 (0.08, 0.19) |

Table IV.2. Mean concentrations (in ppm) of lincomycin in surplus honey (lower, upper 95% confidence limits).

| Treatment | 0 day (on treatment) | 7 days after final treatment | 14 days after final treatment | 20 days after final treatment |
|---|---|---|---|---|
| 200 mg (1x) | 4.37 (2.40, 8.14) | 0.15 (0.10, 0.24) | 0.29 (0.18, 0.47) | 0.38 (0.24, 0.62) |
| 1000 mg (5x) | 6.91 (3.71, 13.48) | 0.48 (0.30, 0.80) | 1.29 (0.76, 2.24) | 1.51 (0.89, 2.63) |
| 0 mg (control) | 0.25 (0.16, 0.40) | 0.15 (0.10, 0.24) | 0.16 (0.10, 0.25) | 0.26 (0.17, 0.42) |

## 2. Target Tissue and Marker Residue

The target tissue is honey. A marker residue is not identified because the regulatory method measures microbiological activity of lincomycin rather than a specific compound.

### 3. Tolerances

Tolerances for residues of lincomycin in honey are not required because residues of lincomycin in honey collected from hives treated with lincomycin hydrochloride are very low. Consistent with the labeling for other products approved for use in honey bees, lincomycin hydrochloride should be fed early in the spring or late in the fall and consumed by the bees before the main honey flow begins to avoid contamination of production honey.

### 4. Withdrawal Period and Milk Discard Time

Complete treatments at least four weeks before main honey flow.

## G. Analytical Method for Residues

### 1. Description of Analytical Method:

The analytical method for the detection of residues of lincomycin in honey used in the residue study is a microbiological assay using an oxytetracycline-resistant strain of *Paenibacillus larvae* (the causative agent of American foulbrood disease of honey bees). This method is found in "Diagnosis of Honeybee Diseases," Shimanuki, H, and Knox, D. A. 2000. US Department of Agriculture, Agricultural Handbook No. AH-690.

### 2. Availability of the Method

A copy of the method is on file at the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855.

## V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to LINCOMIX Soluble Powder:

Not for human use.

## VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that LINCOMIX Soluble Powder, when used according to the label, is safe and effective for the control of American foulbrood (*Paenibacillus larvae*) in honey bees. Additionally, data demonstrate that residues in food products derived from honey bees treated with LINCOMIX Soluble Powder will not represent a public health concern when the product is used according to the label.

## A. Marketing Status:

This product can be marketed over-the-counter (OTC) because the approved labeling contains adequate directions for use by laypersons and the conditions of use prescribed on the label are reasonably certain to be followed in practice.

**B. Exclusivity:**

Under section 573(c) of the Federal Food, Drug and Cosmetic Act (the Act), this approval qualifies for SEVEN years exclusive marketing rights beginning on the date of approval because the new animal drug has been declared a designated new animal drug by FDA under section 573(a) of the Act. The seven years of exclusive marketing rights applies only to the control of American foulbrood (*Paenibacillus larvae*) in honey bees indication for which this supplement is approved.

**C. Supplemental Applications:**

This supplemental NADA did not require a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2)).

**D. Patent Information:**

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.